Bioreoxidation of Uranium from the Oak Ridge Y-12 Site: Microbial Community Structure and Function

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RESEARCH OBJECTIVES

Uranium contamination is an unwanted legacy of the Cold War era. When uranium mining and processing for nuclear weapons and fuel were at their peak, uranium-containing wastes accumulated, resulting in a multitude of contaminated sites around the world. Uranium remediation strategies in recent years have focused on containment, minimizing migration of uranium in groundwater to prevent infiltration into surrounding water courses and potable water supplies.

One promising approach to minimizing uranium migration is to catalyze the reduction of soluble U(VI) to the less soluble U(IV). This process can be accelerated by the action of indigenous microorganisms fuelled through addition of exogenous carbon. Organic carbon addition stimulates biomass and microbial activity in these typically nutrient-poor environments, and has a profound impact on microbial community composition. The focus of our work is to use novel high-density DNA microarray technology to accurately monitor changes in composition of microbial populations during lab and field-scale studies of uranium bioreduction and observed reoxidation/remobilization.

APPROACH

To determine if the remobilization of U(VI) was associated with alterations in microbial populations, we have used a novel high-density oligonucleotide-microarray-based approach, which permits simultaneous monitoring of the dynamics of over 9,000 distinguishable prokaryotic taxa/units (OTUs) (Figure 1). To identify dynamic groups of organisms during biostimulation, we have applied hierarchical clustering methods combined with global graphical representation methods. To validate the high-density array approach, we analyzed identical samples, using a more common clone-library approach in addition to confirmatory tests using quantitative polymerase chain reactions (PCR). This is the first application of high-density array technology in profiling complex microbial communities such as those in soils or sediments.

ACCOMPLISHMENTS

Array analysis of the contaminated sediment confirmed the presence of most clone sequence types detected using conventional methodology. In addition, array analysis also indicated the presence of many bacterial families not detected by cloning, including those of importance for uranium reduction (e.g., *Geobacteraceae*). PCR with primers specific for *Geobacteraceae* confirmed this finding. Array analysis of bacterial communities during a laboratory-scale remediation simulation permitted time- and cost-effective monitoring of the dynamics of over 9,000 groups of bacteria. By using hierarchical clustering and principal component analysis, it was possible to readily identify those organisms responding with treatment or over time.

Lactate infusion into columns resulted in a significant change in bacterial populations, and following an initial period of successful uranium immobilization/reduction, we observed a remobilization of uranium, despite an adequate supply of lactate and suitable redox conditions. However, array data demonstrated that bacteria capable of uranium reduction had not decreased in quantity; therefore, a loss of this functional group was not considered the primary reason for the remobilization of uranium.

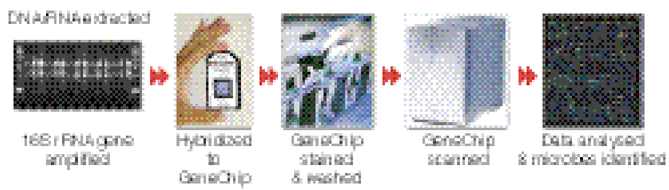

Figure 1. Flow schematic for high-density DNA microarray analysis of environmental microbial communities

SIGNIFICANCE OF FINDINGS

This is the first application of high-density microarrays in analysis of complex microbial communities. We have also demonstrated the ability to accurately track complete populations of bacteria using this array and have shown that loss of microbial functional groups was not a primary cause of uranium remobilization. Bacterially mediated carbonate accumulation has been identified as a possible driver of uranium remobilization, and we are currently investigating further biological and geochemical explanations for this significant observation.

PUBLICATIONS

Wan, J., T.K. Tokunaga, E.L. Brodie, Z. Wang, Z. Zheng, D. Herman, T.C. Hazen, M.K. Firestone, and S.R. Sutton, Reoxidation of bioreduced uranium under reducing conditions. Environmental Science and Technology, 39, 6162-6169, 2005.

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